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## Healthspan and lifespan extension by fecal microbiota transplantation into progeroid mice

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38 The gut microbiome is emerging as a key regulator of several metabolic, immune and  
39 neuroendocrine pathways<sup>1,2</sup>. Gut microbiome deregulation has been implicated in major  
40 conditions such as obesity, type-2 diabetes, cardiovascular disease, non-alcoholic fatty  
41 acid liver disease and cancer<sup>3-6</sup>, but its precise role in aging remains to be elucidated.  
42 Here, we find that two different mouse models of progeria are characterized by intestinal  
43 dysbiosis with alterations that include an increase in the abundance of proteobacteria and  
44 cyanobacteria, and a decrease in the abundance of verrucomicrobia. Consistent with these  
45 findings, we found that human progeria patients also display intestinal dysbiosis and that  
46 long-lived humans (*i.e.*, centenarians) exhibit a substantial increase in verrucomicrobia  
47 and a reduction in proteobacteria. Fecal microbiota transplantation from wild-type mice  
48 enhanced healthspan and lifespan in both progeroid mouse models and transplantation  
49 with the verrucomicrobia *Akkermansia muciniphila* was sufficient to exert beneficial  
50 effects. Moreover, metabolomic analysis of ileal content points to the restoration of  
51 secondary bile acids as a possible mechanism for the beneficial effects of reestablishing  
52 a healthy microbiome. Our results demonstrate that correction of the accelerated aging-  
53 associated intestinal dysbiosis is beneficial, suggesting the existence of a link between  
54 aging and the gut microbiota that provides a rationale for microbiome-based interventions  
55 against age-related diseases.

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57

58 **Keywords:** aging, centenarians, fecal transplants, dysbiosis, longevity, metagenomics,  
59 microbiome, progeria.

60

61 Traditionally seen as detrimental, the pathophysiological implications of the  
62 microbiota have considerably expanded in the last years. It is now known that the  
63 microbiota has essential metabolic and immunological functions conserved from worms<sup>7</sup>  
64 to humans<sup>1,2</sup>. In mammals, the gut microbiota is involved in food processing, activation  
65 of satiety pathways, protection against pathogens, and production of metabolites  
66 including vitamins, short chain fatty acids and secondary bile acids<sup>8-10</sup>. The gut  
67 microbiota also signals to distant organs, contributing to the maintenance of host  
68 physiology<sup>11</sup>. Intestinal microbiota alterations are associated with major conditions like  
69 obesity, type-2 diabetes, cardiovascular disease, non-alcoholic fatty acid liver disease,  
70 cancer, and the response to antineoplastic therapy<sup>3-6</sup>.

71 Although some works have explored the microbiome profile of long-lived  
72 humans<sup>12,13</sup>, no alterations have been described in accelerated aging syndromes. In this  
73 work, we studied the gut microbiome of two mouse models of Hutchinson-Gilford  
74 progeria syndrome (HGPS), patients with HGPS<sup>14</sup> and Nestor-Guillermo progeria  
75 syndrome (NGPS)<sup>15</sup>, as well as human centenarians and their controls. We found  
76 intestinal dysbiosis in both mouse models and progeria patients. In turn, the microbiota  
77 of centenarians is characterized by the presence of both pathological- and health-  
78 associated bacterial genera. We show that fecal microbiota transplantation (FMT) from  
79 wild-type (WT) donors to progeroid recipients attenuates the accelerated-aging  
80 phenotype and increases survival, whereas FMT from progeroid donors to WT recipients  
81 induces metabolic alterations. Analysis of centenarians and progeria mouse models point  
82 to a beneficial role for the genus *Akkermansia*, as oral gavage of *Akkermansia muciniphila*  
83 extends the lifespan of progeroid mice.

84 To explore the relevance of microbiome in progeria, we first studied the gut  
85 metagenome profile of the *Lmna*<sup>G609G/G609G</sup> mouse model of HGPS<sup>16</sup>, by comparing WT

86 and *Lmna*<sup>G609G/G609G</sup> mice at three different ages: 1 month (WT 1mo and *Lmna*<sup>G609G/G609G</sup>  
87 1mo), 4 months –when *Lmna*<sup>G609G/G609G</sup> mice exhibit a progeroid phenotype – (WT 4mo  
88 and *Lmna*<sup>G609G/G609G</sup> 4mo), and 22 months (for WT mice only; WT 22mo; Extended Data  
89 Fig. 1a). To assess how progeria affects the gut microbial community structure, we  
90 studied the alpha- and beta-diversity associated with each genotype, and compared the  
91 microbial diversity within and between communities. Alpha-diversity was analyzed by  
92 calculating the Chao1 (a proxy for community richness) and Shannon’s index (a proxy  
93 for diversity, taking into account both richness and evenness). We did not observe  
94 differences in bacterial diversity or richness within any of the mouse groups (Extended  
95 Data Fig. 1b,c and Supplementary Table 1). Next, we evaluated the beta-diversity across  
96 mouse groups, identifying a differential clustering of *Lmna*<sup>G609G/G609G</sup> 4mo mice in a  
97 principal coordinate analysis (PCoA) using the Bray-Curtis dissimilarity (qualitative  
98 measure) (Fig 1a) and the Jaccard distances (quantitative measure) (Extended Data Fig.  
99 1d). Similar differences were revealed by hierarchical clustering, where *Lmna*<sup>G609G/G609G</sup>  
100 4mo mice were grouped together and separated from all other groups (Extended Data Fig.  
101 1e,f).

102         Next, we calculated the percentage of bacterial taxa in each group (Fig. 1b and  
103 Extended Data Fig. 2a), applying a linear discriminant analysis (LDA) effect size (LEfSe)  
104 method (see Methods; Supplementary Table 2). We noted a similar profile for WT and  
105 *Lmna*<sup>G609G/G609G</sup> mice at 1 month of age (Fig. 1b and Extended Data Fig. 2a), observing  
106 solely an increment in the genera *Allobaculum* from the family Erysipelotrichaceae,  
107 *Desulfovibrio* (class deltaproteobacteria) and Clostridiales of the families  
108 Ruminococcaceae and Lachnospiraceae, in *Lmna*<sup>G609G/G609G</sup> 1mo (Extended Data Fig. 2b).  
109 As WT mice aged (from 1 to 4 months), we noted an increment in genera such as  
110 *Allobaculum*, *Ruminococcus*, *Coprococcus*, *Turicibacter* or *Parabacteroides* (Extended

111 Data Fig. 2c); however, the changes from 1 to 4 months were more profound in progeroid  
112 mice, displaying a loss in *Akkermansia* and *Dehalobacterium* and an enrichment in  
113 *Parabacteroides*, *Prevotella* and the Enterobacteriaceae, among other differences  
114 (Extended Data Fig. 2d). When comparing WT and progeroid mice at 4 months of age,  
115 the pattern was substantially different (Fig. 1b,c and Extended Data Fig. 2a), in agreement  
116 with the progression of the aging phenotype. The main differences were a reduction in  
117 abundance of Erysipelotrichales (phylum Firmicutes), Burkholderiales (class  
118 Betaproteobacteria) and Verrucomicrobiales (phylum Verrucomicrobia) in progeroid  
119 mice, together with an increase in Bacteroidales (phylum Bacteroidetes),  
120 Deferribacterales (phylum Deferribacteres), YS2 (phylum Cyanobacteria) and the  
121 proteobacteria Enterobacteriales and Pseudomonadales (class Gammaproteobacteria)  
122 (Fig. 1c and Supplementary Table 2). At a lower taxonomical level, *Lmna*<sup>G609G/G609G</sup> 4mo  
123 mice showed a loss in Clostridiaceae, *Allobaculum*, *Sutterella*, *Dehalobacterium*,  
124 *Rikenella* and *Akkermansia* (Fig. 1d). Of note, high abundance of *Akkermansia* has been  
125 associated with improved immunomodulation and metabolic homeostasis, reduced  
126 inflammation, and protection against atherosclerosis<sup>17-19</sup>. By contrast, *Lmna*<sup>G609G/G609G</sup>  
127 4mo exhibited an increment in *Mucispirillum*, *Enterococcus*, *Acinetobacter*,  
128 *Staphylococcus*, *Parabacteroides*, *Bacteroides*, *Prevotella* and Enterobacteriaceae,  
129 which contains the genus *Escherichia* and has been associated with dysbiosis and  
130 intestinal inflammation<sup>20</sup> (Fig. 1d). Changes in *Escherichia* and *Akkermansia* were  
131 validated afterwards at the species level by qPCR in a second, independent group of  
132 *Lmna*<sup>G609G/G609G</sup> mice (Fig. 1e). The gut microbiome of a fraction of WT 22mo exhibited  
133 a tendency towards intestinal dysbiosis (Extended Data Fig. 2a); however, the only  
134 significant shift consisted in the loss in *Rikenella* (Extended Data Fig. 2e), a change also  
135 found when comparing *Lmna*<sup>G609G/G609G</sup> 4mo to WT 4mo mice (Fig. 1d). To explore the

136 functional implications of the microbiome shift in progeroid mice, we then investigated  
137 the metagenome data with PICRUSt and HUMAnN2 (see Methods). LEfSe analysis  
138 detected an increase in KEGG pathways related to pathological bacteria such as ‘Bacterial  
139 invasion of epithelial cells’ (ko05100) and ‘Flagellar assembly’ (ko02040), and a  
140 differential enrichment in multiple metabolic pathways (Extended Data Fig. 2f and  
141 Supplemental Table S3).

142 To confirm the association of gut dysbiosis with progeria, we analyzed the gut  
143 microbiota of a different progeria model, *Zmpste24*<sup>-/-</sup> mice<sup>21</sup> (Extended Data Fig. 3a).  
144 Although *Zmpste24*<sup>-/-</sup> mice showed no differences in bacterial diversity (Extended Data  
145 Fig. 3b), they exhibited higher bacterial richness (Extended Data Fig. 3c). Like  
146 *Lmna*<sup>G609G/G609G</sup> mice, *Zmpste24*<sup>-/-</sup> animals showed differences in beta diversity at the  
147 quantitative (Fig. 1f) and qualitative level (Extended Data Fig. 3d), clustering differently  
148 from WT mice, and exhibited signs of dysbiosis (Fig. 1g, Extended Data Fig. 3e) with a  
149 high abundance of Proteobacteria (class Alphaproteobacteria) and Cyanobacteria (Fig.  
150 1g, Extended Data Fig. 3e and Supplementary Table 2). *Zmpste24*<sup>-/-</sup> mice also showed a  
151 tendency to a lower amount of Verrucomicrobia (Extended Data Fig. 3e), although this  
152 trend was less marked than in *Lmna*<sup>G609G/G609G</sup> animals, perhaps due to the later onset of  
153 the progeroid phenotype in *Zmpste24*<sup>-/-</sup> mice. At a lower taxonomic level, we found,  
154 among other differences, an enrichment in the order YS2 (phylum Cyanobacteria) and  
155 the genera *Bacteroides*, *Parabacteroides* and *Prevotella* (Fig. 1h), similarly to  
156 *Lmna*<sup>G609G/G609G</sup> 4mo mice.

157 To investigate the possible existence of gut dysbiosis in human progeria, we  
158 obtained fecal samples from four children with HGPS and their healthy siblings, as well  
159 as from a patient with NGPS and his healthy sibling and mother (Extended Data Fig. 4a).  
160 When comparing progeria patients with their healthy controls, we found no differences

161 in alpha-diversity (Extended Data Fig. 4b,c) or beta-diversity indices (Fig. 2a and  
162 Extended Data Fig. 4d). However, when samples were compared by geographical  
163 location (*i.e.*, comparing families), a differential clustering was observed at the  
164 quantitative (Fig. 2b) and qualitative levels (Extended Data Fig. 4e). Despite the low  
165 number of samples, these results suggest that the environment has more influence on the  
166 gut microbiome than the pathological condition, which is in accordance with recent  
167 findings<sup>22,23</sup>. Yet, in each of the studied families, the progeria patients had a different  
168 profile compared to their healthy siblings. This was particularly evident in families where  
169 more than one healthy member was studied (Extended Data Fig. 4f). LEfSe analysis  
170 pointed to a loss in *Gemmiger* –family Ruminococcaceae– and an enrichment in  
171 *Clostridium* from families Erysipelotrichaceae and Lachnospiraceae in progeria patients  
172 (Fig. 2c).

173         Considering the gut microbiome alterations observed in mice and progeria  
174 patients, we hypothesized that individuals with exceptionally long lifespans (*i.e.*,  
175 centenarians) might possess a health-promoting microbiome. Therefore, we performed a  
176 metagenomic analysis of a centenarian cohort and ethnically-matched healthy adult  
177 controls (Extended Data Fig. 5a). Centenarians showed lower alpha-diversity compared  
178 to their controls (Extended Data Fig. 5b), and a considerably lower bacterial richness  
179 (Extended Data Fig. 5c). Also, both groups clustered differentially, based on quantitative  
180 (Fig. 2d) and qualitative (Extended Data Fig. 5d) beta-diversity indexes, indicating that  
181 centenarians had a different microbial community structure than controls. Indeed, we  
182 encountered distinct microbial profiles in both groups (Fig. 2e and Extended Data Fig.  
183 5e). LEfSe analysis showed that centenarians presented less Betaproteobacteria and more  
184 Synergistia and Verrucomicrobiae, among others (Fig. 2e and Supplementary Table 2).  
185 At a lower taxonomic level, centenarians were characterized by a lower abundance of



186 Desulfovibrionaceae, Lachnospiraceae and Erysipelotrichaceae – the last two enriched in  
187 progeria patients – and *Prevotella*, *Sutterella*, *Roseburia*, or *Butyrivibrio*, among other  
188 differences (Fig. 2f). Notably, centenarians exhibited a higher abundance of  
189 Enterobacteriaceae, Ruminococcaceae and Christensenellaceae and the genera  
190 *Klebsiella*, *Lactobacillus*, *Parabacteroides* and *Akkermansia* (Fig. 2f). These results are  
191 in line with previous studies reporting high levels of *Akkermansia* and  
192 Christensenellaceae and low levels of *Roseburia* in centenarians<sup>12,24</sup>.

193         Based on the aforementioned results, we hypothesized that changes in the gut  
194 microbiota might accompany the accelerated aging of HGPS mice. To explore this  
195 possibility, we performed FMT in four different mouse groups. First, we used WT control  
196 mice as microbiota donors (herein referred to as WTmic, *WT microbiota*) and  
197 *Lmna*<sup>G609G/G609G</sup> mice as recipients. We also transplanted *Lmna*<sup>G609G/G609G</sup> mice with  
198 microbiota from older *Lmna*<sup>G609G/G609G</sup> mice, that is, progeroid mice with a more advanced  
199 phenotype (oG609Gmic, *old Lmna*<sup>G609G/G609G</sup> microbiota) (Fig. 3a). FMT effectiveness  
200 was evaluated by comparing the gut metagenomic profiles of control, transplanted and  
201 donor mice, showing that transplanted progeroid mice acquired the donor microbiota  
202 (Extended Data Fig. 6a,b). Additionally, we performed microbiome ablation and sham-  
203 transplanted *Lmna*<sup>G609G/G609G</sup> mice (herein referred to as EmptyT, *empty transplant*) (Fig.  
204 3a). *Lmna*<sup>G609G/G609G</sup>-WTmic manifested a delayed loss of body weight and temperature  
205 (Fig. 3b,c). Phenotype-dependent hypoglycemia was avoided and renal perivascular  
206 fibrosis was attenuated in *Lmna*<sup>G609G/G609G</sup>-WTmic (Fig. 3d,e). Spleen weight, typically  
207 reduced in progeroid mice, was similar in *Lmna*<sup>G609G/G609G</sup>-WTmic and *Lmna*<sup>G609G/G609G</sup>  
208 controls, but lower in *Lmna*<sup>G609G/G609G</sup>-oG609Gmic (Extended Data Fig. 6c). We also  
209 noted an increase in intestinal inflammation markers in *Lmna*<sup>G609G/G609G</sup> mice that was  
210 recovered in *Lmna*<sup>G609G/G609G</sup>-WTmic (Extended Data Fig. 6d). Surprisingly, some

211 markers were also decreased in *Lmna*<sup>G609G/G609G</sup>-oG609Gmic, pointing to a possible  
212 beneficial effect of the FMT protocol *per se* (Extended Data Fig. 6d). Most importantly,  
213 *Lmna*<sup>G609G/G609G</sup>-WTmic showed improved survival compared to control *Lmna*<sup>G609G/G609G</sup>  
214 mice ( $P = 0.0029$ ), with a 13.5% increase in median lifespan (160 vs 141 days,  
215 respectively) (Fig. 3f). *Lmna*<sup>G609G/G609G</sup>-WTmic also exhibited an extended maximal  
216 survival (9% increment,  $P = 0.04$ ) (Fig. 3f and Extended Data Fig. 6e). In contrast,  
217 *Lmna*<sup>G609G/G609G</sup>-oG609Gmic showed reduced survival compared to control  
218 *Lmna*<sup>G609G/G609G</sup> mice ( $P = 0.045$ ), with a reduction in median lifespan (129 days) (Fig.  
219 3f). *Lmna*<sup>G609G/G609G</sup>-EmptyT did not show survival differences when compared to control  
220 *Lmna*<sup>G609G/G609G</sup> mice (Extended Data Fig. 6f). Lastly, to evaluate the potential  
221 pathogenicity of progeroid microbiota, we also performed FMT from old *Lmna*<sup>G609G/G609G</sup>  
222 donors into WT mice (WT-oG609Gmic). Effective gut colonization was validated by  
223 comparing the metagenomic profiles of control, transplanted and donor mice (Extended  
224 Data Fig. 6a,b). We did not observe progeria-related features in WT-oG609Gmic mice.  
225 However, this maneuver caused metabolic alterations, including a higher body weight,  
226 higher glucose levels, lower O<sub>2</sub> consumption, lower CO<sub>2</sub> production and reduced energy  
227 expenditure (Fig. 3g-k and Extended Data Fig. 7a-d).

228 To validate these results, we also performed FMT in *Zmpste24*<sup>-/-</sup> mice using  
229 microbiota from WT mice (*Zmpste24*<sup>-/-</sup>-WTmic). This manipulation caused *Zmpste24*<sup>-/-</sup>  
230 mice to manifest a less pronounced cervicothoracic lordokyphosis, larger body size and  
231 maintained grooming (Fig. 3l). Body weight loss (Fig. 3m and Extended Data Fig. 8a)  
232 and hypoglycemia (Fig. 3n) were avoided in *Zmpste24*<sup>-/-</sup>-WTmic mice. Like  
233 *Lmna*<sup>G609G/G609G</sup> mice transplanted with WT microbiota, *Zmpste24*<sup>-/-</sup>-WTmic mice also  
234 exhibited enhanced survival ( $P = 0.0092$ ), with a median lifespan increase of 13.4% (279

235 vs 246 days) (Fig. 3o). Maximum survival was increased by almost 8% ( $P = 0.09$ ) (Fig.  
236 3o and Extended Data Fig. 8b).

237 The results above in both human and murine gut metagenomic profiles described  
238 a loss in *A. muciniphila* in *Lmna*<sup>G609G/G609G</sup> 4mo mice and an increase in centenarians'  
239 samples. Since this bacterium exerts beneficial effects in other models<sup>17,19,25,26</sup>, we tested  
240 if external supply of *A. muciniphila* to *Lmna*<sup>G609G/G609G</sup> mice would improve their  
241 healthspan or lifespan. By supplementing *Lmna*<sup>G609G/G609G</sup> mice with *A. muciniphila*  
242 through oral gavage, we obtained a modest lifespan extension ( $P = 0.016$ ) (Fig. 4a),  
243 suggesting a protective role of this microorganism against accelerated aging  
244 manifestations. As previously described<sup>19</sup>, *A. muciniphila* supplementation induced ileal  
245 expression of *Reg3g* (Fig. 4b) and favored the thickening of the intestinal mucosa layer  
246 (Fig. 4c). *Lmna*<sup>G609G/G609G</sup> mice receiving *A. muciniphila* also showed an increment in the  
247 intestinal trefoil factor *Tff3* (Fig. 4b), which might promote wound healing and repair of  
248 the mucosa layer<sup>27</sup>.

249 Finally, to investigate the potential mechanisms accounting for the healthspan and  
250 lifespan extension of *Lmna*<sup>G609G/G609G</sup> mice after FMT, we performed metabolome  
251 profiling of ileal content from WT, *Lmna*<sup>G609G/G609G</sup> and *Lmna*<sup>G609G/G609G</sup>-WTmic mice.  
252 Significant changes were analyzed by metabolite set enrichment analysis using KEGG  
253 pathways, detecting an enrichment in 'secondary bile acid biosynthesis' (Fig. 4d). As  
254 recently described<sup>28</sup>, different bile acids were decreased in *Lmna*<sup>G609G/G609G</sup> compared to  
255 WT mice, and recovered in *Lmna*<sup>G609G/G609G</sup>-WTmic (Fig. 4e). Moreover, *Lmna*<sup>G609G/G609G</sup>  
256 mice ileal content exhibited a depletion of the monosaccharides arabinose and ribose, the  
257 nucleoside inosine, and the ether-phospholipid PCae (18:0) that was reversed upon FMT  
258 with WT microbiota (Fig. 4f).

259 FMT might modulate obesity and metabolism in humans and mice<sup>29,30</sup>, ameliorate  
260 metabolic syndrome in patients<sup>31</sup>, and contribute to the treatment of refractory immune  
261 checkpoint inhibitor (ICI)-associated colitis<sup>32</sup> and recurrent infections by *Clostridium*  
262 *difficile*<sup>33</sup>. The promising *in vivo* results obtained in this study suggest that therapeutic  
263 interventions on the intestinal microbiome may lead to healthspan end even lifespan  
264 improvements. In this regard, we show that *A. muciniphila* administration lead to a  
265 lifespan enhancement in progeria, thus extending previous findings on pro-health  
266 activities of *Akkermansia* spp. in the intestinal tract of mammals<sup>17-19</sup> and in aged mice<sup>34</sup>.  
267 Previous studies have described that successful FMT treatment of recurrent infection by  
268 *C. difficile* relies on restoration of correct bile acid metabolism<sup>35,36</sup>, and that antibiotic-  
269 induced microbiome depletion reduces the secondary bile acid pool<sup>37</sup>. Of note, secondary  
270 bile acids are produced by the gut microbiota<sup>38</sup> and positively correlate with *Akkermansia*  
271 levels in mice<sup>39</sup>. Considering that bile acids regulate metabolism and anti-inflammatory  
272 signals<sup>40</sup> and that they are depleted in *Lmna*<sup>G609G/G609G</sup> mice<sup>28</sup>, the restoration of  
273 secondary bile acids – and other metabolites (arabinose, ribose, inosine) – by FMT might  
274 contribute to extend healthspan and lifespan in progeroid mice (Fig. 4g). Future work  
275 might identify the functional mechanisms by which some bacterial species and  
276 metabolites are responsible for the healthspan and lifespan extension conferred by FMT,  
277 and explore the applicability of FMT in normal or accelerated aging.

278

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310 data. M.T.F.-G. performed histopathological analysis. N.S. and A.M.N. performed the  
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316

317

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319

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422 **Figure legends**

423 **Figure 1. Gut dysbiosis in *Lmna*<sup>G609G/G609G</sup> and *Zmpste24*<sup>-/-</sup> progeroid mice.** (a)  
424 Principal coordinates analysis (PCoA) of beta-diversity using the Bray-Curtis  
425 dissimilarity metric among samples of the five groups of mice analyzed ( $P = 0.001$ ;  
426 PERMANOVA). G609G 4mo mice show statistical differences with each of the other  
427 groups (see **Supplementary Table 1**). Each dot represents an individual mouse. PCo1  
428 and PCo2 represent the percentage of variance explained by each coordinate (WT 1mo,  
429  $n = 6$ ; G609G 1mo,  $n = 5$ ; WT 4mo,  $n = 8$ ; G609G 4mo,  $n = 9$ ; WT 22mo,  $n = 8$ ). (b)  
430 Average relative abundance of prevalent microbiota at the class level in the 5 groups  
431 studied: 1-month-old wild-type (WT 1mo,  $n = 6$ ), 1-month-old *Lmna*<sup>G609G/G609G</sup> (G609G  
432 1mo,  $n = 5$ ), adult 4-month-old wild-type (WT 4mo,  $n = 8$ ), 4-month-old *Lmna*<sup>G609G/G609G</sup>  
433 (G609G 4mo,  $n = 9$ ) and 22-month-old wild-type (WT 22mo,  $n = 8$ ). The low abundance  
434 bacteria group includes all bacterial classes with less than 0.5% of total abundance. (c)  
435 Taxonomic cladogram obtained from LEfSe analysis showing bacterial taxa (phylum,  
436 class and order) that were differentially abundant in progeroid and WT mice at 4 months  
437 of age. Red indicates increased abundance in progeroid mice, grey indicates increased  
438 abundance in WT mice. Def: Deferribacteres (phylum), Deferribacteres (order),  
439 Deferribacterales (class); Cya: Cyanobacteria (phylum); Pseudo: Pseudomonadales;  
440 Entero: Enterobacteriales; Burkho: Burkholderiales. (d) Results of LEfSe analysis  
441 showing bacterial taxa that were significantly different in abundance between G609G and  
442 WT mice at 4 months of age. For c and d: WT 4mo,  $n = 8$ ; G609G 4mo,  $n = 9$ . (e)  
443 Validation using qPCR of the differences in abundance of *E. coli* ( $W = 6$ ,  $P = 0.0046$ )  
444 and *A. muciniphila* ( $W = 52$ ,  $P = 0.03$ ) between WT ( $n = 8$ ) and G609G mice ( $n = 8$ ) at  
445 10 weeks of age. Two-tailed unpaired Wilcoxon rank-sum test. Each dot represents an  
446 individual mouse. In the box plots, upper and lower hinges correspond to the first and  
447 third quartiles, center line represents the median, whiskers indicate the highest and lowest  
448 values that are within  $1.5 * IQR$ , and data beyond the end of the whiskers are outliers and  
449 plotted as points. (f) PCoA of beta-diversity using the Bray-Curtis dissimilarity metric  
450 between *Zmpste24*<sup>-/-</sup> and WT mice ( $P = 0.003$ , PERMANOVA). Each dot represents an  
451 individual mouse. PCo1 and PCo2 represent the percentage of variance explained by each  
452 coordinate. (g) Average relative abundance of prevalent microbiota at the class level in  
453 WT and *Zmpste24*<sup>-/-</sup> mice. (h). LEfSe analysis showing bacterial classes that were  
454 significantly different in abundance between *Zmpste24*<sup>-/-</sup> and WT mice. For f-h: 4-month-

455 old WT mice, n = 8 (same as panel a-d); 4-month-old *Zmpste24*<sup>-/-</sup> mice, n = 4. p: phylum;  
456 c: class; o: order; f: family; g: genus.

457

458 **Figure 2. Alterations in the gut microbiome in progeria patients and long-lived**  
459 **humans. (a,b)** PCoA of beta-diversity using the Bray-Curtis dissimilarity metric among  
460 fecal samples analyzed by (a) health status ( $P = 0.5$ , PERMANOVA) (Control, n = 9  
461 individuals; Progeria, n = 5 individuals) and (b) family ( $P = 0.001$ , PERMANOVA)  
462 (Family A, n = 3; Family B, n = 3; Family C, n = 3; Family D, n = 2; Family E, n = 3).  
463 Each dot represents one person. PCo1 and PCo2 represent the percentage of variance  
464 explained by each coordinate. (c). Results of LEfSe analysis showing bacterial genera  
465 whose abundance significantly differed between progeria patients and their healthy  
466 siblings (Control=9 individuals; Progeria=5 individuals). (d) PCoA of beta-diversity  
467 using the Bray-Curtis dissimilarity metric of healthy controls (HC; n = 14 individuals)  
468 and centenarians (Ce; n = 17 individuals) ( $P = 0.001$ , PERMANOVA). Each dot  
469 represents one person. (e) Average relative abundance of prevalent microbiota at the class  
470 level in centenarians (Ce; n = 17 individuals) and healthy controls (HC; n = 14  
471 individuals). The low abundance bacteria group includes all bacterial classes with less  
472 than 0.5% of total abundance. (f) Results of LEfSe analysis showing bacteria, at the  
473 lowest taxonomic level, that were significantly different in abundance in centenarians  
474 (Ce; n = 17 individuals) vs healthy controls (HC; n = 14 individuals). p: phylum; c: class;  
475 o: order; f: family; g: genus.

476

477 **Figure 3. Effects of fecal microbiota transplantation in progeroid and WT mice (a)**  
478 Scheme of the experimental design, in which the effects of FMT were assessed using 4  
479 different groups of progeroid mice: control untransplanted *Lmna*<sup>G609G/G609G</sup> mice (n = 11),  
480 *Lmna*<sup>G609G/G609G</sup> mice transplanted with fecal microbiota from WT mice (*Lmna*<sup>G609G/G609G</sup>-  
481 WTmic; n = 11), *Lmna*<sup>G609G/G609G</sup> mice transplanted with microbiota from older  
482 *Lmna*<sup>G609G/G609G</sup> mice (*Lmna*<sup>G609G</sup>-oG609Gmic; n = 11) and *Lmna*<sup>G609G/G609G</sup> mice  
483 subjected to ablation of their own microbiota and transplanted with empty buffer  
484 (*Lmna*<sup>G609G</sup>-EmptyT; n = 8). Transplants were carried on at ~8-10 weeks of age, using as  
485 donors 4 months-old WT and 4 months-old *Lmna*<sup>G609G/G609G</sup> mice. (b) Comparison of the  
486 percentage of initial body weight between *Lmna*<sup>G609G/G609G</sup> (n = 11) and *Lmna*<sup>G609G/G609G</sup>-  
487 WTmic (n = 11) mice over the indicated time period. Differences in body weight over

488 time were assessed with a linear mixed model and analyzed with an Anova Type II Wald  
489 Chi-square test ( $X^2 = 8.06$ ,  $df = 1$ ,  $P = 0.0045$ ). Data are presented as mean  $\pm$  SEM. (c)  
490 Box plots showing differences in the body temperature between WT ( $n = 4$ ),  
491 *Lmna*<sup>G609G/G609G</sup> ( $n = 6$ ) and *Lmna*<sup>G609G/G609G</sup>-WTmic ( $n = 5$ ) mice. One-way ANOVA  
492 with Tukey's correction ( $F = 20.45$ ,  $df = 2$ ,  $P = 0.0001$ ). Exact adjusted p-values are  
493 reported within the plot. (d) Comparison of the glucose levels between *Lmna*<sup>G609G/G609G</sup>  
494 ( $n = 6$ ) and *Lmna*<sup>G609G/G609G</sup>-WTmic ( $n = 5$ ) mice over the indicated time period. Unpaired  
495 two-tailed Student's *t*-test (for week 5:  $t = 2.74$ ,  $df = 36$ ,  $P = 0.009$ ). (e) Representative  
496 histological images of renal vasculature, showing increased perivascular fibrosis in  
497 *Lmna*<sup>G609G/G609G</sup> mice ( $n = 10$ ) (blue staining indicated with an arrow) compared to WT  
498 ( $n = 10$ ) and *Lmna*<sup>G609G/G609G</sup> mice transplanted with WT microbiota (*Lmna*<sup>G609G</sup>-WTmic;  
499  $n = 8$ ). Renal fibrosis scores were analyzed with a Kruskal-Wallis test with Dunn's  
500 correction. Exact adjusted p-values are reported within the plot. Each dot represents a  
501 single mouse. The horizontal line represents the mean  $\pm$  95% of confidence interval (CI).  
502 Scale bar = 100  $\mu$ m. (f) Percentage survival of *Lmna*<sup>G609G/G609G</sup> ( $n = 11$ ), *Lmna*<sup>G609G/G609G</sup>-  
503 WTmic ( $n = 11$ ) and *Lmna*<sup>G609G/G609G</sup> mice transplanted with old *Lmna*<sup>G609G/G609G</sup>  
504 microbiota (*Lmna*<sup>G609G</sup>-oG609Gmic;  $n = 11$ ). Differences were analyzed with the Log-  
505 rank Mantel-Cox test and BH correction was applied after pairwise comparisons between  
506 all experimental groups, including Empty transplant (Extended Data Fig. 6f). Hazard ratio  
507 (HR) was calculated using a Cox proportional model. For *Lmna*<sup>G609G/G609G</sup>-WTmic vs  
508 *Lmna*<sup>G609G/G609G</sup>, HR of 0.2 [95% confidence interval (CI) 0.07–0.53],  $P = 0.0012$ ; for  
509 *Lmna*<sup>G609G/G609G</sup>-oG609Gmic vs *Lmna*<sup>G609G/G609G</sup>, HR of 4.1 [95% CI 1.5–11.1],  $P =$   
510 0.005. Median and maximal survival, percentage of median and maximal lifespan  
511 extension and log-rank test adjusted p-values are indicated in the Kaplan-Meier plot.  
512 Transplantation was performed starting at ~8-10 weeks of age. (g) Comparison of body  
513 weight between male WT mice transplanted with progeroid microbiota (WT-  
514 oG609Gmic;  $n = 7$ ) and male WT controls ( $n = 7$ ) over the indicated time period.  
515 Differences of body weight over time were assessed with a linear mixed model and  
516 analyzed with an Anova Type II Wald Chi-square test ( $X^2 = 5.49$ ,  $df = 1$ ,  $P = 0.019$ ). Data  
517 is presented as mean  $\pm$  SEM. (h) Blood glucose levels of WT mice ( $n = 14$ ; 7 males and  
518 7 females) and WT-oG609Gmic ( $n = 14$ ; 7 males and 7 females). Unpaired two-tailed  
519 Student's *t*-test ( $t = 4.56$ ,  $df = 26$ ,  $P = 0.0001$ ). Each dot represents a single mouse. The  
520 horizontal line represents the mean  $\pm$  95% CI. (i-k) Metabolic parameters, measured with

521 an Oxymax system, of WT (n = 14; 7 males and 7 females) and WT-oG609Gmic (n = 14;  
522 7 males and 7 females) mice. Differences were analyzed with an unpaired two-tailed  
523 Welch's *t*-test. **(i)** Volume of O<sub>2</sub> consumed (VO<sub>2</sub>) (t = 2.71, df = 28, *P* = 0.011). **(j)**  
524 Volume of CO<sub>2</sub> produced (VCO<sub>2</sub>) (t = 3.94, df = 23, *P* = 0.0006). **(k)** Energy expenditure  
525 (EE) (t = 3.15, df = 25, *P* = 0.0043). **(l)** Representative pictures of control *Zmpste24*<sup>-/-</sup>  
526 mice and *Zmpste24*<sup>-/-</sup> mice transplanted with WT microbiota (*Zmpste24*<sup>-/-</sup>-WTmic).  
527 Transplanted mice appear to be healthier, as manifested by an ameliorated cervicothoracic  
528 lordokyphosis, a larger size and better grooming. **(m)** Body weight at 35 weeks of life of  
529 *Zmpste24*<sup>-/-</sup> mice (n = 4) and *Zmpste24*<sup>-/-</sup>-WTmic mice (n = 6). Unpaired two-tailed  
530 Student's *t*-test (t = 4.31, df = 8, *P* = 0.0026). **(n)** Blood glucose levels of *Zmpste24*<sup>-/-</sup> mice  
531 (n = 5) and *Zmpste24*<sup>-/-</sup>-WTmic mice (n = 7) at 30 weeks. Unpaired two-tailed Student's  
532 *t*-test (t = 5.47, df = 11, *P* = 0.0002). **(o)** Percentage survival of *Zmpste24*<sup>-/-</sup> (n = 7) and  
533 *Zmpste24*<sup>-/-</sup>-WTmic female mice (n = 7). Differences were performed with the Log-rank  
534 Mantel-Cox test (*P* = 0.0092). HR of 0.15 [95% CI 0.03-0.75], *P* = 0.021. Median and  
535 maximal survival, percentage of median and maximal lifespan extension and exact *p*-  
536 value are indicated in the Kaplan-Meier plot. Transplantation was performed starting at  
537 ~7-10 weeks of age. For c, d, i-k, m and n: box plots show upper and lower hinges  
538 corresponding to the first and third quartiles, center line represents the median, whiskers  
539 indicate the highest and lowest values that are within 1.5 \* IQR, and data beyond the end  
540 of the whiskers are outliers and plotted as points. Each dot represents a single mouse.

541

542 **Figure 4. *Akkermansia muciniphila* supplementation in progeroid mice and**  
543 **metabolomic analysis of ileal content.** **(a)** Percent survival of *Lmna*<sup>G609G/G609G</sup> mice  
544 receiving *A. muciniphila* by oral gavage (AKK; n = 9) compared to *Lmna*<sup>G609G/G609G</sup> mice  
545 (G609G; n = 12). *A. muciniphila* transplantation was performed starting at 7-8 weeks of  
546 age. Differences in survival were analyzed with Log-rank Mantel-Cox test (*P* = 0.0163).  
547 Hazard ratio (HR) was calculated using a Cox proportional model (HR of 0.31 [95%  
548 confidence interval (CI) 0.11–0.86], *P* = 0.0244). **(b)** Relative expression levels of *Reg3g*  
549 and *Tff3* in G609G (n = 8) and AKK (n = 7) mice. Differences were analyzed with an  
550 unpaired two-tailed Student's *t*-test. For *Reg3g*, t = 2.33, df = 13, *P* = 0.0365; for *Tff3*, t  
551 = 2.42, df = 13, *P* = 0.0306. Data are represented as dots (one per mouse) with mean ±  
552 95% of CI. **(c)** Left, representative histological images of the intestinal mucosa layer of  
553 G609G and AKK mice. Arrow indicates the thickness of the mucosa layer. Scale bar =

554 100  $\mu\text{m}$ . Right, comparison of mucosa layer thickness of G609G (n = 8) and AKK (n =  
555 7) mice. Differences were calculated using an unpaired two-tailed Welch's *t*-test ( $t = 2.72$ ,  
556  $df = 12$ ,  $P = 0.018$ ). Data are represented as dots (one per mouse) with mean  $\pm$  95% of  
557 CI. For b and c, G609G group was composed by 4 males and 4 females, and AKK group  
558 was composed by 4 males and 3 females. **(d)** Metabolic set enrichment analysis of all  
559 metabolites with statistically significant differences between the three groups: WT (n =  
560 8), *Lmna*<sup>G609G/G609G</sup> (n = 8) and *Lmna*<sup>G609G/G609G</sup>-WTmic (n = 8), using all annotated KEGG  
561 pathways (see Methods). **(e)** Box plots showing the relative levels of different bile acids  
562 in WT (n = 8), *Lmna*<sup>G609G/G609G</sup> (n = 8), and *Lmna*<sup>G609G/G609G</sup>-WTmic (n = 8). CA: cholic  
563 acid; CDCA: chenodeoxycholic acid;  $\beta$ MCA: beta-muricholic acid; DCA: deoxycholic  
564 acid; HCA: hyocholic acid;  $\omega$ MCA: omega-muricholic acid; UDCA: ursodeoxycholic  
565 acid; 12-KCDCA: 12-ketochenodeoxycholic acid. **(f)** Box plots showing the relative  
566 levels of selected metabolites between WT (n = 8), *Lmna*<sup>G609G/G609G</sup> (n = 8) and  
567 *Lmna*<sup>G609G/G609G</sup>-WTmic (n = 8). For e and f, each group of 8 mice was composed by 4  
568 males and 4 females. In both panels, differences were analyzed using a one-way ANOVA  
569 with multiple comparison test with one-side relative to control *Lmna*<sup>G609G/G609G</sup> mice.  
570 Exact adjusted p-values are reported in each plot. In the box plots, upper and lower hinges  
571 correspond to the first and third quartiles, center line represents the median, whiskers  
572 indicate the highest and lowest values that are within 1.5 \* IQR, and data beyond the end  
573 of the whiskers are outliers and plotted as points. Each dot represents a single mouse. **(g)**  
574 Schematic representation of the model proposed in this work. WT mice are characterized  
575 by a gut symbiosis in which bacterial metabolites participate in systemic homeostasis. By  
576 contrast, progeroid mice are characterized by a gut dysbiosis, leading to decreased bile  
577 acid levels and a reduced healthspan and lifespan. FMT with WT microbiota (WT mic)  
578 into progeroid mice raises bile acids levels and improves healthspan and lifespan.

579

580

## 581 **Methods**

### 582 *Mouse models*

583 Both *Lmna*<sup>G609G/G609G</sup> and *Zmpste24*<sup>-/-</sup> mice were generated by crossing  
584 *Lmna*<sup>G609G/+</sup> and *Zmpste24*<sup>+/-</sup> mice and genotyped in our laboratory as previously  
585 described<sup>16,21</sup>. All mice used in this study were in C57BL/6N background. Mice were  
586 caged separately by sex and transplantation group and checked daily for water and food  
587 availability, as well as for good physical condition. Mice were housed in cages with solid  
588 floors, sawdust and nests. Mice in all groups were given every day pellets of food  
589 previously softened in water for 1–2 hours to facilitate the feeding of progeroid mice. All  
590 components of the cages, including food, had been previously autoclaved. For glucose  
591 determination, blood samples were obtained from the tail vein and measured with Accu-  
592 Chek glucometer (Roche Diagnostics; Mannheim, Germany). Body temperature was  
593 measured by rectal probe (Acorn® Temp TC Thermocouple Thermometer, Fisher  
594 Scientific; Hampton, NH). Transplantation experiments in progeroid mice began at ~6-  
595 10 weeks of age. In the *Lmna*<sup>G609G/G609G</sup> survival experiments, 11 mice were analyzed in  
596 control (7 males and 4 females), transplanted with WT (5 males and 6 females) and  
597 transplanted with *Lmna*<sup>G609G/G609G</sup> groups (5 males and 6 females), whereas 8 mice (3  
598 males and 5 females) were analyzed in the empty transplant group. *Zmpste24*<sup>-/-</sup>  
599 transplantation experiments were performed with 7 females per group. In the WT  
600 transplantation experiments, 14 animals per group were used (7 males and 7 females). In  
601 the WT and *Lmna*<sup>G609G/G609G</sup> validation experiment, 8 mice (4 males and 4 females) were  
602 used in each group of transplanted mice, and pooled samples from 12 WT (6 males and 6  
603 females) and 15 *Lmna*<sup>G609G/G609G</sup> mice (8 males and 7 females) were used as donors.  
604 Survival curves were analyzed with Log-rank (Mantel-Cox) test. Maximum survival was  
605 analyzed by Fisher's exact test at 80<sup>th</sup> percentile<sup>41</sup>. All animal experiments were approved

606 by the Committee for Animal Experimentation of the Universidad de Oviedo (Spain) and  
607 performed in accordance with the European and Spanish legislative and regulatory  
608 guidelines (European convention ETS 1 2 3, on the use and protection of vertebrate  
609 mammals in experimentation and for other scientific purposes, and Spanish Law 6/2013,  
610 and R.D. 53/2013 on the protection and use of animals in scientific research), making  
611 every effort to minimize mouse discomfort.

#### 612 *Human samples*

613 We obtained samples from four HGPS families and one NGPS family that include  
614 individuals affected with progeria and their healthy siblings. Additionally, we collected  
615 samples from a Spanish cohort composed by 17 centenarians, independently of their  
616 health status, and 14 healthy ethnically-matched adults, aged 30–50 years and with no  
617 history of any major disease. Research involving humans was approved by the Ethical  
618 Committee of Regional Clinical Research of the Principality of Asturias, project  
619 no.105/16. All participants read and signed an informed consent.

#### 620 *Preparation of 16S DNA for metagenome profiling*

621 Mouse feces were collected for 4 h and immediately kept at -80 °C until DNA  
622 extraction. For human studies, samples were picked and stored at -20 °C in OmniGene  
623 Gut kits (Ref. OMR-200, DNA Genotek; Ora Sure Technologies; Bethlehem, PN). In  
624 all cases (mice and human samples), DNA was extracted using the PowerSoil DNA  
625 Isolation kit (MO BIO Laboratories, Quiagen N.V.; Hilden, Germany). DNA quality and  
626 quantification were assessed with Qubit fluorometer (Thermo Fisher Scientific; Waltham,  
627 MA). Libraries were prepared following the 16S Metagenomic Sequencing Library  
628 Preparation protocol from Illumina. Briefly, the region V3-V4 from 16S rRNA was  
629 amplified using the primers 341F/805R to which Illumina Sequencing adapters and dual-

630 index barcodes of the Nextera XT kit were added (FWD 5'-  
631 TCGTCGGCAGCGTCAGATGTGTATAAG AGACAGCCTACGGGNGGCWGCAG-  
632 3' and REV 5'-GTCTCGTGGGCTCGGAGA  
633 TGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'). Sequencing was  
634 performed in a MiSeq platform (IMEGEN, Valencia, Spain) using 2 x 300 bp-end  
635 protocol.

### 636 *Metagenome profiling*

637 Raw paired-end reads were processed with QIIME 2 (version 2018.6.0). Sequence  
638 quality controls were performed with DADA2 (qiime dada2 denoise-paired, with a  
639 number of expected errors higher than 6): reads were filtered, trimmed, denoised,  
640 dereplicated, forward and reverse sequences were merged, and chimeras were removed.  
641 Taxonomy was assigned using a pre-trained Naïve Bayes classifier, with a trimmed  
642 version of Greengenes 13\_8 99% OTUs, which includes the V3-V4 regions, bounded by  
643 the 341F/805R primer pair. For *Lmna*<sup>G609G/G609G</sup> model, we obtained 3,071,354 paired-  
644 end reads of 300 nucleotides each, with 1,084 OTUs identified after quality filtering  
645 (Extended Data Fig 1a), and for *Zmpste24*<sup>-/-</sup> model, we obtained 1,149,187 paired-end  
646 reads of 300 nucleotides each, with 882 OTUs identified after quality-filtering (Extended  
647 Data Fig 3a). 4-month-old C57BL/6N WT mice in experiments from Figure 1 were used  
648 as controls for both *Lmna*<sup>G609G/G609G</sup> and *Zmpste24*<sup>-/-</sup> mice. For human samples in progeria  
649 patients, we obtained 1,709,578 paired-end reads of 300 nucleotides each, with 691 OTUs  
650 identified after quality filtering (Extended Data Fig 4a), whereas for centenarians and  
651 healthy controls we obtained 6,196,891 paired-end reads of 300 nucleotides each, with  
652 1,761 OTUs identified after quality-filtering (Extended Data Fig 5a). For validating FMT  
653 experiments 8 mice per group and condition were used, obtaining 8,761,200 paired-end  
654 reads of 300 nucleotides each, with 18,402 OTUs identified after quality filtering. Alpha



655 diversity and statistical analysis were calculated based on different metrics (shannon,  
656 chao1). Beta diversity was measured using Bray-Curtis dissimilarity and Jaccard  
657 similarity index (braycurtis, jaccard). Samples were hierarchical clustered with the  
658 Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method and using  
659 different beta diversity metrics (braycurtis, jaccard). Differences in bacteria abundance  
660 were calculated using LEfSe<sup>42</sup>. Metagenome functional content prediction was performed  
661 using PICRUST<sup>43</sup> and HUMAnN2 (v0.11.1)<sup>44</sup>, and analyzed with LEfSe.

### 662 *Microbiome transplants*

663         Prior to transplantation, mice were treated for three consecutive days with 200  $\mu$ L  
664 of an antibiotic-cocktail (with each daily dose being administered by oral gavage after a  
665 6-hour fast) which contained 1 g/L ampicillin, 0.5 g/L neomycin, 0.5 g/L vancomycin and  
666 1 g/L metronidazole. Thereafter mice were given 100  $\mu$ L of the microbiome suspension  
667 twice a week for 2 weeks, starting the first day after the antibiotic cycle. After this 2-week  
668 period mice received the microbiome suspension once a week until natural death or  
669 sacrifice. For the microbiome suspension preparation, 2-5 fresh feces pellets (80-100 mg)  
670 were resuspended with a vortex in 600  $\mu$ L of reduced PBS (PBS with 0.5 g/L cysteine  
671 and 0.2 g/L Na<sub>2</sub>S). After resuspension, tubes containing the feces in reduced PBS were  
672 centrifuged at 2,500 rpm (500 g) for 1 min to remove insolubilized material, and 100  $\mu$ L  
673 of supernatant were administered to the mice by oral gavage. Empty transplant group  
674 received the same antibiotics treatment and were transplanted only with reduced PBS.

### 675 *Calorimetry measurements*

676         Metabolic parameters (oxygen consumption, carbon dioxide production and total  
677 energy expenditure), were obtained using the comprehensive lab animal monitoring  
678 system (Oxymax CLAMS, Columbus Instruments; Columbus, OH) and analyzed

679 following the manufacturer's instructions. Mice were monitored for 48 h and the first 24  
680 h were discarded in the analysis, considering them as acclimation period.

#### 681 *Akkermansia muciniphila* culture and oral supplementation

682 Cultures of the strain *Akkermansia muciniphila* CIP107961 grown for 24 h in  
683 GAM medium (Nissui Pharmaceutical Co; Tokyo, Japan) supplemented with 0.25%  
684 (w/v) L-cysteine (Sigma Chemical Co.; St. Louis, MO) (GAMc) in anaerobic conditions  
685 were used to inoculate (2% v/v) fresh pre-reduced GAMc broth which was incubated for  
686 24 h. Afterwards, cultures were washed with PBS and concentrated in anaerobic PBS that  
687 included 25% (v/v) glycerol to a concentration of about  $1 \times 10^{10}$  cfu/mL under strict  
688 anaerobic conditions and stored at  $-80$  °C until use. To test the viability of glycerol  
689 stocks, serial dilutions in PBS were made and deep plated on agar-GAMc. Plates were  
690 incubated under anaerobic conditions for 5 days to determine the *Akkermansia* counts  
691 (cfu/ml). Before administration by oral gavage, the glycerol stocks were thawed under  
692 anaerobic conditions and diluted with anaerobic PBS to a final concentration of  $2 \times 10^8$   
693 viable cfu/0.1 mL. *Lmna*<sup>G609G/G609G</sup> mice were treated by oral gavage with 100  $\mu$ L of either  
694 *Akkermansia* suspension (AKK group, n = 9) or anaerobic PBS (control group, n=12)  
695 three days a week beginning at 12 weeks of age and until decease.

#### 696 *Quantitative polymerase chain reaction*

697 For RNA expression analysis, total RNA from about 30 mg of frozen ileon  
698 samples was extracted using Trizol (Life Technologies) and resuspended in nuclease-free  
699 water (Life Technologies). 1–2  $\mu$ g of total RNA was used for reverse transcription using  
700 the QuantiTect Reverse Transcription kit (Quiagen N.V.).  $10 \times$  diluted cDNA was used  
701 for quantitative polymerase chain reaction (qPCR) using Power SYBR Green PCR  
702 Master Mix (Life Technologies; Carlsbad, CA) and Real-Time PCR (7300 HT, Applied

703 Biosystems; Foster City, CA). Gene expression was normalized to the GAPDH  
704 expression. For bacterial quantification, DNA from mouse feces was extracted as  
705 described above. 1– 4 ng of DNA were used for qPCR reactions using specific primers to  
706 amplify bacterial 16S rDNA. Bacterial abundance was assessed by normalizing with the  
707 abundance of total bacteria in feces using the conserved eubacterial 16S rDNA primer  
708 pair UniF340/UniR514. Results are represented as relative quantification using RQ value  
709 ( $RQ=2^{-\Delta\Delta C_t}$ ). Primer sets for qPCR analyses are shown in Supplementary Table 4.

### 710 *Metabolomic analysis*

711 Prior to sacrifice for sample collection, mice were starved overnight and thereafter  
712 allowed to eat for 4 h. 30 mg of ileum content for each condition were first weighted and  
713 solubilized into 1 mL polypropylene Precellys lysis tubes, with 1 mL of cold lysate buffer  
714 (MeOH/Water/Chloroform, 9/1/1, -20 °C). After being vortexed for 10 min, samples were  
715 centrifuged (10 min at 15,000 g, 4 °C), and the upper phase was collected and split in two  
716 parts: the first 270 µL used for the Gas Chromatography coupled to Mass Spectrometry  
717 (GC-MS) and 250 µL used for the Ultra High Pressure Liquid Chromatography coupled  
718 to Mass Spectrometry (UHPLC-MS). For GC-MS measurements, 150 µL from the  
719 aliquot were transferred to a glass tube and evaporated. Then, 50 µL of methoxyamine  
720 (20 mg/mL in pyridine) was added to dried extracts and samples were then stored at room  
721 temperature in the dark for 16 h. The day after, 80 µL of MSTFA was added and final  
722 derivatization carried out at 40 °C during 30 min. Samples were then transferred in vials  
723 and directly injected into GC-MS. For LC-MS measurements, the collected supernatant  
724 was evaporated in microcentrifuge tubes at 40 °C in a pneumatically-assisted concentrator  
725 (Techne DB3, Techne; Staffordshire, UK). Dried extracts were solubilized with 450 µL  
726 of MilliQ water and aliquoted in 3 microcentrifuge tubes (100 µL) for each LC method

727 and one microcentrifuge tube for safety. Aliquots were transferred to LC vials and  
728 injected into LC-MS or kept at -80 °C until injection. A daily qualification of the  
729 instrumentation was set up with automatic tune and calibration processes. These  
730 qualifications were completed with double injections of standards mixes, at the beginning  
731 and at the end of the run, as for a blank extracted sample to control the background  
732 impurities. Mixtures were adapted for each chromatographic method. After the  
733 extraction, fractions of each biological sample were pooled to create a Quality Control  
734 (QC) sample, use to passivate the column before the analysis with the proper biological  
735 matrix. This QC sample was re-injected in each batch to monitor and correct analytical  
736 bias. Analytical methods and data processing were performed as previously described<sup>45</sup>.  
737 Results were represented as the normalized area of the MS picks in log<sub>2</sub> scale using  
738 arbitrary units. Normalization was performed by correcting the area of the MS picks  
739 across the batches using the QC pooled samples and by centering their values around the  
740 mean of the QC areas. Standard reagents (acetonitrile, methanol, chloroform, acetic acid  
741 and dibutylamine acetate concentrate) were acquired from Sigma Aldrich (Saint Luis,  
742 MO). Differentially expressed metabolites in each condition were identified using  
743 moderate t-statistic implemented in the R/Bioconductor package limma<sup>46</sup>, using sex as a  
744 covariate. Metabolites with a nominal *P*-value < 0.05 and q-value < 0.25 were selected  
745 for metabolic set enrichment analysis using one-sided Fisher's exact test against all  
746 metabolites annotated in each KEGG pathway. Metabolomic results are provided in  
747 Supplementary Table 5.

#### 748 *Histological analysis*

749 Kidneys and intestines were fixed in 4% paraformaldehyde in PBS and stored in  
750 50% ethanol. Fixed tissues were embedded in paraffin by standard procedures. Blocks  
751 were sectioned (5 μm) and stained with hematoxylin and eosin and Masson trichrome

752 (H&E, MT, kidney) and periodic acid Schiff-alcian blue (PAS-AB, intestine). Renal  
753 perivascular fibrosis was analyzed/graded from 0 to 4, by using a histology damage score  
754 (0: no lesion; 1: focal lesion; 2: multifocal mild lesion; 3: multifocal moderate lesion; 4:  
755 diffuse, moderate or severe damage). Five fields were scored from each slide.

#### 756 *Statistical analysis*

757         Number of mice allocated per group was based on previous experiments and their  
758 distribution was randomized, being indicated in each Figure legend. Comparisons  
759 between two groups following normal distribution were performed using a two-tailed  
760 Student's *t*-test, while one-factor analysis of variance (ANOVA) was used for  
761 comparisons of three or more groups. Unless specified in the Figure legends, adjusted *p*-  
762 values were obtained using Tukey's correction. For non-parametric distributions, the  
763 Wilcoxon rank-sum and Kruskal-Wallis test (the latter followed by the Dunnett *post-hoc*  
764 test) were performed for comparisons between two groups or three or more groups,  
765 respectively. Survival analysis was performed by using the Kaplan-Meier method and  
766 statistical differences were analyzed with the Log-rank (Mantel-Cox) test (GraphPad  
767 Prism 6.0 and survival R package). Body weight curves were analyzed using a linear-  
768 mixed effect model (lme4 R package). The hazard ratio was calculated using a Cox  
769 proportional hazards regression model (survival R package). Sample sizes for lifespan  
770 experiments were chosen with a power of 80%, based on our previous studies<sup>16,28,47</sup>.  
771 Maximal survival was calculated using Fisher's test at 80<sup>th</sup> percentile. Plots were  
772 generated with GraphPad Prism 6.0 and RStudio (using ggplot2 R package), and edited  
773 with Illustrator CC (21.0.0). Statistical analysis was performed using RStudio and  
774 GraphPad Prism 6.0. Exact P-values are indicated in each figure.

#### 775 *Reporting summary*

776 Further information on research design is available in the Nature Research Life  
777 Sciences Reporting Summary linked to this article.

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#### 779 **Data availability statement**

780 Sequence data supporting these findings have been deposited in EGA under with  
781 accession number EGAS00001003656. Metabolomics data are provided in the  
782 Supplementary Table 5. Any additional data generated and analyzed in this study are  
783 available from the corresponding authors upon reasonable request.

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